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PROGRESS REPORT

THE INCIDENCE OF THE PINEWOOD NEMATODE IN A SOUTHERN PINE BEETLE INFESTATION IN CENTRAL LOUISIANA

Prepared by:

D. N. Kinn 9-5-85
D. N. Kinn
Research Entomologist

Approved by:

Peter L. Lorio, Jr. 9-6-85
Peter L. Lorio, Jr. Date
Project Leader

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Progress Report Summary
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A large southern pine beetle (*Dendroctonus frontalis* Zimm.) infestation located in central Louisiana was surveyed for the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle. Pinewood nematodes were present in 4.2 percent of 94 trees attacked by *D. frontalis* and were absent from all of 101 unattacked trees surrounding the infestation. Sequential sampling of beetle infested trees at monthly intervals revealed an increase in the incidence of nematodes as the season progressed. Samples taken from different heights on beetle infested boles did not differ significantly in the incidence of worms.

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D. N. Kinn

Enclosed is a manuscript draft to be used as the progress report.

THE INCIDENCE OF THE PINEWOOD NEMATODE[1]

IN A SOUTHERN PINE BEETLE[2] INFESTATION IN CENTRAL LOUISIANA[3]

D. N. Kinn

USDA Forest Service, Southern Forest Experiment Station,

Pineville, Louisiana 71360

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1 *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae)

2 *Dendroctonus frontalis* (Coleoptera: Scolytidae)

3 Received for publication:

ABSTRACT

A large southern pine beetle (Dendroctonus frontalis Zimm.) infestation located in central Louisiana was surveyed for the pinewood nematode, Bursaphelenchus xylophilus (Steiner and Buhrer) Nickle. Pinewood nematodes were present in 4.2 percent of 94 trees attacked by D. frontalis and were absent from all of 101 unattacked trees surrounding the infestation. Sequential sampling of beetle infested trees at monthly intervals revealed an increase in the incidence of nematodes as the season progressed. Samples taken from different heights on beetle infested boles did not differ significantly in the incidence of worms.

Key Words: Pinewilt Disease, Bursaphelenchus xylophilus, Dendroctonus frontalis, Cerambycidae

Pine wilt disease was first reported from Japan in 1913. The disease is now a major threat to Japan's forest industry and in 1980 the Japanese government allocated $35 million to control the disease (Kondo, et al. 1982). The disease is probably caused by a phytotoxin produced by the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer 1934) Nickle 1970 (Bolla, et al. 1982), a metabolite of an associated bacterium (Oku, et al. 1980), or both.

The disease appears to be endemic in the U.S., not epidemic as in Japan (Dropkin, et al. 1981). *Bursaphelenchus xylophilus* was originally described from specimens collected in Louisiana and is probably native to this continent since many North American *Pinus* spp. exhibit some degree of resistance to the disease (Mamiya 1983). However, it is a potential threat, especially in areas planted with exotic pines or native pines planted on off-site conditions (Wingfield, et al. 1980). Of 30 pine species tested by Futai and Furuno (1979), *Pinus elliottii* Engelm. var. *elliotti* (slash pine) and *P. taeda* L. (loblolly pine) were found to be highly resistant to pine wilt disease. However, resistance varies inversely with the number of nematodes in the inoculum.

In Japan the nematode is vectored by a cerambycid, *Monochamus alternatus* Hope. The major vectors in the United States are the long horn beetles: *Monochamus carolinensis* (Olivier), *M. scutellatus* (Say) and *M. titillator* (F) (Linit, et al. 1983, Williams 1980, Wingfield 1983). The dauerlarvae leave the beetle and enter wounds made by long horn beetles while feeding on succulent bark (Kondo, et al 1982). Inoculations made in this manner are considered primary. Nematodes can also be introduced into dead or dying trees through ovipositional pits produced by female longhorn beetles. In the latter case the trees may have been killed or stressed by fungal disease agents, insects or physical factors. Inoculations made during oviposition are considered secondary (Wingfield 1983).

This study was undertaken to determine the incidence of *B. xylophilus* in an active southern pine beetle (*Dendroctonus frontalis* Zimm.) infestation and to note any changes in the incidence of the nematode infections through time.

MATERIALS AND METHODS

A large southern pine beetle infestation located 6.8 km (4.5 mi.) NW of Williana, Louisiana in the Kisatchie National Forest (Saddle Bayou area) was used for this study. An initial survey for the pinewood nematode was made in July, 1983 by sampling 94 loblolly pine trees that had been or were currently under attack by the southern pine beetle as well as 101 uninfested trees located just outside of the advancing edge of the beetle infestation. Samples were taken at a height of 1 m using a brace and a 1.9 cm (0.75 in.) auger bit to drill a 3.8 cm (1.5 in.) deep hole into the xylem. Before sampling, the outer bark and phloem-cambium interface were removed with a chisel. Borings were collected in Ziploc® * bags and held in an ice-chest until processed. The wood borings were wrapped in Kimwipes® * and soaked in distilled water for 18 to 24 hours in the laboratory. Temporary slide mounts were made of any nematodes found in the water. When only immature aphelencoidid worms were present, pieces of xylem and individual worms were transferred to fungal cultures in an attempt to rear the worms to adults. *Monilinia fructicola* (Wint.) Honey (brown rot of stone fruits) was used to culture the worms.

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* Use of trade, firm, or corporation names is for the reader's information and convenience. Such use does not constitute official endorsement or approval by the U.S. Department of Agriculture of any product or service to the exclusion of others that may be suitable.

A total of 34 southern pine beetle infested trees not previously sampled were climbed between July 27 and Oct. 24 to determine the distribution of *B. xylophilus* along the bole. The bark beetle infested bole was divided into five equal length sections and xylem samples were taken from each height using a brace and bit. Sampling heights varied among trees but ranged between 156 and 165 cm. All but five of these trees were climbed again 20 days after the initial visit and another set of samples taken from each height. One set of eight trees was sampled on 3 occasions (Sept. 7, 27, Oct. 21). The first samples were taken when bark beetle oviposition was complete but before reemergence. The second samples were taken when the southern pine beetles were pupae or brood adults. A χ^2 test was used to test the independence of sample height and presence of pinewood nematodes.

RESULTS AND DISCUSSION

Only 4 of the 94 bark infested trees (4.2%) in the initial survey revealed the presence of pinewood nematodes and none of the 101 samples from uninfested trees contained this worm. Of the 34 trees climbed, 38% eventually became infected with *B. xylophilus*. Pinewood nematodes were found in 5.8% of these trees on the first sampling date and in 27.6% on the second sampling date (Table 1). *B. xylophilus* was absent from two sections on one tree and one section on another that had tested positive for worms on the first sampling date. None of the 8 trees sampled 3 times were infected on the first sampling date, 4 tested positive for pinewood nematodes on the second visit and 7 contained *B. xylophilus* on the third sampling date. The increase may be the result of the time it takes for *B. xylophilus* to migrate through the tree or represent the introduction of more nematodes through additional beetle oviposition. Although it has been reported that *B. xylophilus* feeds on blue

stain fungi (Wingfield, et al. 1984), blue stain (Ceratocystis sp.) was associated with pinewood nematodes in only 18.4% of the positive samples.

No significant differences were noted in the incidence of worms among the 5 sample heights (x^2, P> 0.05). In Japan B. xylophilus is more numerous in the upper portion of the trunk in trees wilting in September and is more uniformly distributed on the bole in trees wilting in March (Kanehori, et al. 1975).

The incidence of pinewood nematodes may have been higher than that indicated in this study since some fungus cultures did not produce worms and others developed high populations of Rhabdontolaimus frontali Massey 1974. When present in our samples, R. frontali appeared to reproduce more rapidly than B. xylophilus and tended to suppress the numerical increase of B. xylophilus.

The absence of pinewood nematodes in healthy trees surrounding the southern pine beetle infestation, the low density of the worms in trees killed by beetles prior to July, and the lower incidence of the worm on the first sampling date (2 of 34 trees) in comparison to the second sampling date (8 of 29 trees) in those trees sampled between July and October indicate that the trees probably were not stressed by pine wilt disease prior to being attacked by bark beetles. The rapid increase in the size of this infestation precludes the possibility that most of the trees were asymptomatically stressed by pine wilt disease prior to attack by bark beetles. Wingfield, et al. (1982) found pinewood nematodes only in trees or portions of trees killed by pathogenic fungi or insects. It has also been suggested that Ips beetles found in B. xylophilus infected trees in Indiana may have been responsible for killing the trees (Marshall and Favinger 1980). Nematodes were most likely introduced into these dying and dead trees by ovipositing cerambycids. Transmission of

this worm to dead and dying trees is an efficient means of perpetuating the population until vectored to healthy host trees (Wingfield 1983). Secondary infestations such as that at Saddle Bayou may also provide a reservoir in which cerambycids and pinewood nematodes reproduce and from which infected sawyers may migrate, vectoring pine wilt disease to healthy stands, nursery plantations, or logs in saw mills.

The spotty distribution of *B. xylophilus* along the bole and the failure of finding worms from sample heights previously found to be infected, indicate the need for better sampling procedures and the need for additional studies on the epidemiology of the disease in conifers native to North America.

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REFERENCES CITED

Bolla, R. I., F. Shaheen, and R. E. K. Winter 1982. Phytotoxin production in <u>Bursaphelenchus xylophilus</u> pine wilt. J. Nematol. 14(4): 431.

Dropkin, V. H., A. Foudin, E. Kondo, M. Linit, M. Smith and K. Robbins 1981. Pinewood nematode: A threat to U.S. forests? Plant Disease 65(12): 1022-1027.

Futai, K. and T. Furuno 1979. The variety of resistances among pine species to pine wood nematode, <u>Bursaphelenchus lignicolus</u>. Bull. Kyoto Univ. For. 51: 23-26.

Kanehori, F., K. Yasui and S. Ono 1975. Studies on the ecology and eradication of pine-bark beetles (1) Changes of <u>Bursaphelenchus</u> sp. and adults and larvae of pine-bark beetles in wood. Sci. Repts. Faculty Agricult., Okayama Univ. 46: 72-78.

Kondo, E., A. Foudin, M. Linit, M. Smith, R. Bolla, R. Winter and V. Dropkin 1982. Pine wild disease: nematological, entomological, biochemical investigations. Univ. Missouri-Columbia, Agricult. Exp. Sta. Publ. SR 282. 56 pp.

Linit, M. J., E. Kondo and M. T. Smith 1983. Insects associated with the pinewood nematode, <u>Bursaphelenchus xylophilus</u> (Nematoda: Aphelenchoididae), in Missouri. Environ. Entomol. 12(2): 467-470.

Mamiya, Y. 1983. Pathology of the pine wilt disease caused by <u>Bursaphelenchus xylophilus</u>. Ann. Rev. Phytopathol. 21: 201-220.

Marshall, P. T. and J. J. Favinger 1980. Indiana USA pine wilt nematode <u>Bursaphelenchus lignicolus</u> survey. Proc. Ind. Acad. Sci. 90(0): 254-258.

Oku, H., T. Shiraishi, S. Ouchi, S. Kurozumi and H. Ohta 1980. Pine wilt toxin, the metabolite of a bacterium associated with a nematode. Naturwissenshaften 67: 198-199.

Stephen, F. M. and H. A. Taha 1976. Optimiztion of sampling effort for within-tree populations of southern pine beetle and its natural enemies. Environ. Entomol. 5: 1001-1007.

Williams, D. J. 1980. Pinewood nematode (Bursaphelenchus lignicolus). Iowa, Coop. Plant Pest Rep. 5(33): 627.

Wingfield, M. J. 1983. Transmission of pine wood nematode to cut timber and girdled trees. Plant Disease 67: 35-37.

Wingfield, M. J., R. A. Blauchette, and T. J. Nicholls 1984. Is the pine wood nematode an important pathogen in the United States? J. Forest. 82: 232-235.

Wingfield, M. J., R. A. Blanchette, T. H. Nicholls and K. Robbins 1980. The pine wood nematode: a comparison of the situation in the United States and Japan. Can. J. For. Res. 12(1): 71-75.

_____ 1982. Association of pine wood nematode with stressed trees in Minnesota, Iowa, and Wisconsin. Plant Disease 66(10): 934-937.

Table 1 -- Percent of bark beetle infested trees infected with pine wood nematodes on successive sampling dates.

Dates	No. sampled	First	Second	Third
July	5	20	--	--
July - Aug.	5	0	20	--
Aug.- Sept. 7	10	10	20	--
Sept. 7,27 - Oct.	8	0	50	87
Sept. 27 - Oct.	6	0	17	--